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Electrochemical Properties of Cariogenic Microorganisms and Antibacterial Effect of TiO_2 upon Light Irradiation

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Electrochemical properties of various cariogenic oral microorganisms have been found to depend on kinds of microorganisms. Specific adsorptive behavior of microorganisms to ITO and TiO_2 has been found. Antibacterial effects of TiO_2 upon light irradiation are different with kinds of microorganisms and the type of TiO_2.

KEYWORDS : antibacterial effect, photocatalyst, electrochemistry, TiO_2, microorganisms

口腔内細菌の電気化学的性質と光照射下の酸化チタンによる抗菌効果

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数種類の口腔内う蝕原性細菌と感染性細菌（７種のグラム陽性菌と一種のグラム陰性菌）の電気化学的特性及びそれら細菌に対する酸化チタン(TiO_2)微粒子の抗菌性を明らかにした。供し菌体の酸化還元電位は0.１－1.２Ｖの範囲であった。これらの細菌はITOやTiO_2に特異的な吸着現象を示す。TiO_2添加は電解溶液中では陽極での電位抑制に関係し、細菌の生菌数を減少させた。この光照射した場合の抗菌作用は細菌の種類、TiO_2の種類に依存する。

By studies of electrochemical characteristics of microorganisms, the mechanism of the electrode reaction has been discussed, because it is of fundamental interest.[1-6] The electrochemical properties, because the electrochemical signals of the bacteria can be used for detection of the kind of cell existing in the test solution and its concentration, resulting in particle applications such as bioelectrochemical sensors.[1] In the early works[4] interest was concentrated on one of leaven, Sacchromyces cerevisiae.

On the other hand, studies of photocatalytic reaction in microorganisms revealed that the antibacterial effect of TiO_2 powder was dependent upon a photocatalytic reaction which decreased co-enzyme-A in the microorganisms,[4,7,8] the pH value of the electrolyte solution, and the ability of Streptococcus mutans (S. mutans) to adsorb to TiO_2 powder.

Since S. mutans is well known to exist in the mouth of the mammalian and result in decayed teeth in the human mouth, the effect of TiO_2 on S. mutans under light irradiation is thought to be important for preventing tooth decay. Rcently, Fujishima and co-workers[9] reported the antibacterial effect of TiO_2 powder against to Escherichia coli, and the photoirradiation effects of TiO_2 has been discussed based on photoelectrochemical reaction at the surface of the semiconductor particle.

In the present paper, the electrochemical response of oral bacteria except for S. mutans and communicable bacteria strains have been studied in an aqueous electrolyte solution, and the effect of two types of TiO_2 against to oral bacteria has also been discussed.

Microorganisms used were Streptococcus mitis (S. m), Streptococcus sanguis (S. s), Streptococcus faceium (S. f), Actinomyces viscosus (A. v), Lactobacillus acidophilus (L. a), Lactobacillus casei (L. c), Escherichia coli (E. c) and Staphylococcus aureus (St. a). The semiconductor TiO_2 (concentraiton: 0.1% W/V) particles used were rutile (TP-3; 1.44 μm in diameter, 4.2 specific gravity, Fuji Titanimu Co., Japan) and anatase (ST-41; 50 nm in diameter, 3.9 specific gravity, Ishihara Industrial Co. Ltd, Japan) types.

The electrochemical characteristics of each microorganisms were measured by cyclic voltammetry utilizing potentiostat and function generator (Hokuto Denko Co. Ltd, Japan) with sweep rate 20 mV/sec. A conventional three-electrode cell (reference electrode: Ag/AgCl electrode, counter electrode: a Pt plate, working electrode: In-Sn oxide; ITO) was used. These electrodes have apparent surface area of 1.0 cm^2. After culture of each microorganisms at 37 ℃ for 48 hr in trypticase soy broth (TSB), these bacterial cells were suspended in phosphorous buffer (PB: pH7) and centrifuged (3000 rpm for 15 min). The obtained microorganisms were dispersed in the electrolyte solution (PB).

After preculture of each microorganisms, each culture was diluted with 0.9% NaCl solution and 1×10^4 CFU/ml were suspended in the solution containing TiO_2. Culture of the suspensions was carried out under exposure to light for 0. 60, 120

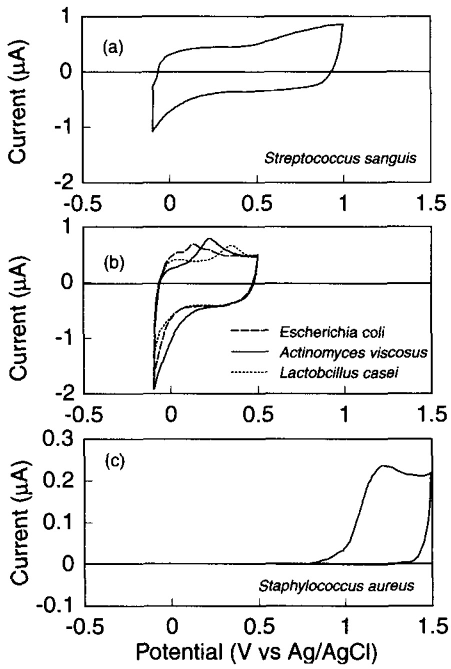

Fig.1. Cyclic voltammograms of ITO electrode on phosphorus buffer solutions containing microorganisms (sweep rate = 20 mV/sec). (a) Streptococcus sanguis, (b) Escherichia coli, Actinomyces viscosus and Lactobcillus casei, (c) Staphylococcus aureus

図1 供試微生物を含む燐酸緩衝液中でのサイクリックボルタモグラム (a) Streptococcus sanguis, (b) Escherichia coli, Actinomyces viscosus and Lactobcillus casei, (c) Staphylococcus aureus and 180 minutes with a fluorescent lamp (wavelength: 578 nm, band width: 202 nm) in an aerobic growth chamber at 37℃ and 55% relative humidity, while being stirred. Samples of the treated suspensions were spread on trypticase soy agar. After 48 hr of incubation at 37℃, the colonies were counted, and bacterial activity was measured with electrochemical methods.

Figure 1 indicates typical cyclic voltammograms of an ITO electrode measured in the phosphorus buffer (PB) solution containing microorganisms. In the microorganisms suspended solution, irreversible anodic waves were observed at 0.1-1.2 V vs Ag/AgCl but not in the blank solution. It should also be mentioned that these anodic waves were also not observed in the microorganisms suspended solutions which were filtrated through a millipore filter (ϕ ; 0.22 μm, Millipore, Nihon Millipore Kogyo Co., Japan) prior to the electrochemical measurement. Therefore, the anodic peak current (Ipa) waves are interpreted to be attributable to electrode active substances existing in the bacterial cell.[7] Since the distance between the electrode surface and the electrode active molecule is required to be shorter than 10 nm for electron transfer between the electrode and the molecule, the electrode active molecule giving the oxidation current should exist on the cell wall of bacteria used.

The electrochemical characteristics of microorganisms are summarized in Table I. The differences of the oxidation potential (Epa) were observed in each microorganisms solution. The highest level of Epa was shown on St. a (1.2 V) and the lowest level of Epa was shown on E. c (0.1 V). Epa of other oral microorganisms was between those of St. a and E. c. As judged from the dissimilar Epa of microorganisms, the different electrode active molecule might be oxidized in each electrolyte solution. The difference in the Epa, which is attributable to a difference in the overvoltage in each irreversible oxidation reaction,

Table1 Electrochemical properties of microorganisms used in this study.

表 1 供試微生物の電気化学的性質

	Ipa (μA)	Epa (mV)
Streptococcus sanguis	0.1	820
Streptococcus faceium	0.6	700
Streptococcus mitis	0.6	430
Lactobacillus acidophilus	0.7	400
Lactobacillus casei	0.8	340
Actionomyces viscosus	1	200
Escherichia coli	0.5	120
Staphylococcus aureus	11	1200

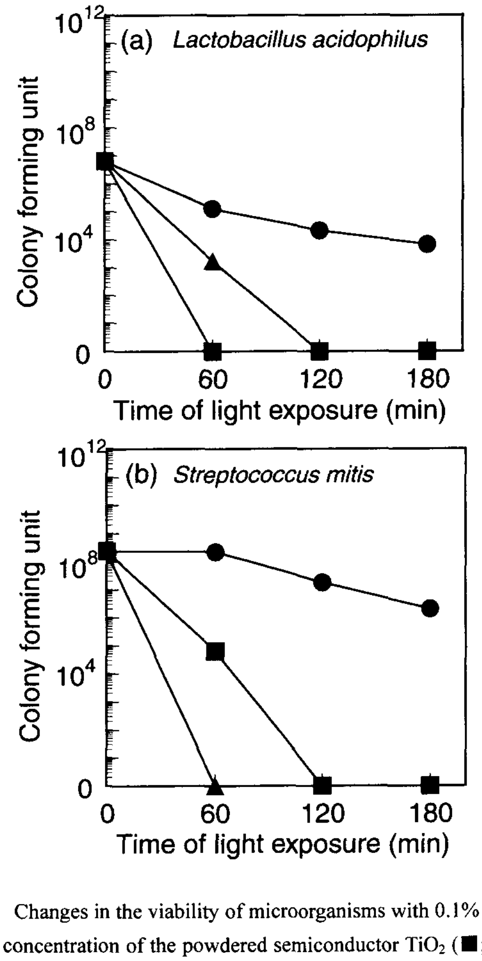

Fig.2. Changes in the viability of microorganisms with 0.1% (W/V) concentration of the powdered semiconductor TiO_2 (■; TP-3, ▲; ST-40) with light exposure time. The controls (●) were incubated without TiO_2. (a) Lactobacillus acidophilus, (b) Streptococcus mitis

図2 光照射下における酸化チタン微粒子0.1%W/V 添加電解液中での供試菌の活性（活動性）の経時変化
酸化チタンの種類(■; TP-3, ▲; ST-40) 酸化チタン無し(●). (a) Lactobacillus acidophilus, (b) Streptococcus mitis

may allow us to classify the microorganisms existing in sample solutions by the electrochemical method. The bacterial strains used in this experiment, i.e.Streptococcus faceium, Streptococcus mitis, Lactobacillus acidophylus, Lactobacillus casei and Actinomyces viscosus, are extremely famous for cariogenic micoroorganisms in the human oral cavity. These bacterial were gram-positive strains. It was assumed that Staphylococcus aurecus and Escherichia coli were one of the typical gram-positive and gram-negative microorganisms, respectively. The correlation between the electrochemical characteristics and kinds of microorganisms and the method to discriminate each microorganisms from electrochemical characteristics are now under study.

On the other hand, the different Ipa of each microorganisms was also shown in Table I. It should also be mentioned that Ipa showed remarkable dependence upon the number of cycles of the cyclic voltammetry, and Ipa was disappeared with the several cycles after suppressing by the number of cycles.[7] This indicates that ITO electrode was covered with bacterial cells adsorbed

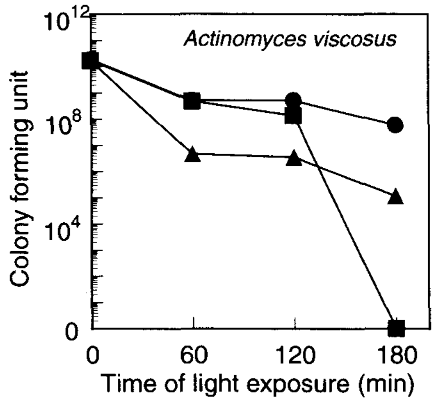

Fig.3. Changes in the viability of Actinomyces viscosus with 0.1% (W/V) concentration of the powdered semiconductor TiO_2 (■; TP-3, ▲; ST-40) with light exposure time. The controls (●) were incubated without TiO_2.

図3 光照射下における酸化チタン微粒子0.1%W/V 添加電解液中での供試菌 Actinomyces viscosus の活性（活動性）の経時変化 酸化チタンの種類(■; TP-3, ▲; ST-40) 酸化チタン無し(●).

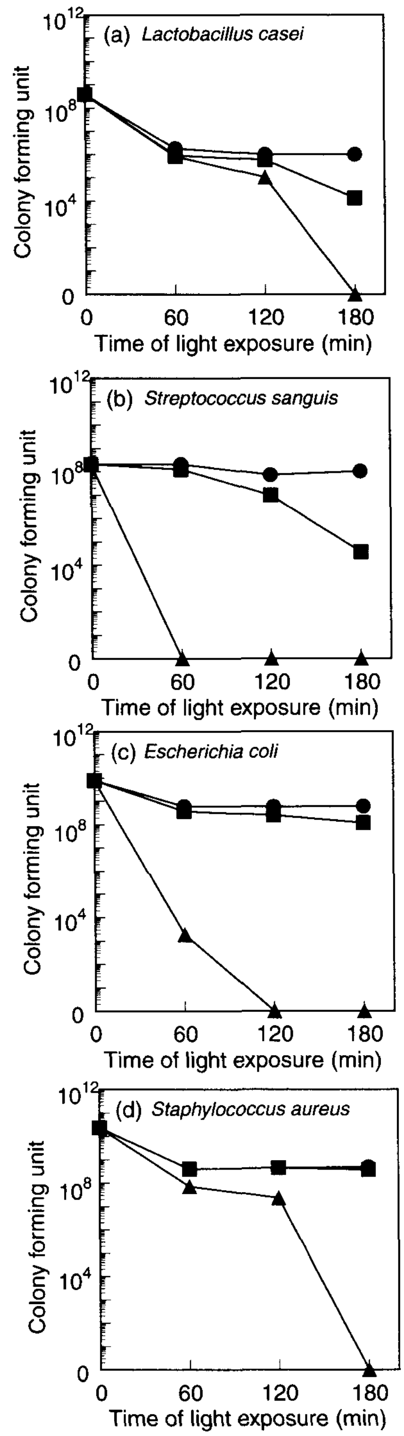

Fig.4. Changes in the viability of microorganisms with 0.1% (W/V) concentration of the powdered semiconductor TiO_2 (■; TP-3, ▲; ST-40) with light exposure time. The controls (●) were incubated without TiO_2. (a) Lactobacillus casei, (b) Streptococcus sanguis, (c) Escherichia coli and (d) Staphylococcus aureus

図4 光照射下における酸化チタン微粒子0.1%W/V 添加電解液中での供試菌の活性（活動性）の経時変化 酸化チタンの種類 (■; TP-3, ▲; ST-40) 酸化チタン無し (●). (a) Lactobacillus casei, (b) Streptococcus sanguis, (c) Escherichia coli and (d) Staphylococcus aureus on the surface.[7] In this report, the same findings with a scanning electron microscope were shown. Therefore, a selective adsorbing nature of the microorganisms might result onto the ITO electrode in the cyclic voltammetry.

We speculate at this stage that the specific activity of ITO in the electrochemical oxidation reaction of microorganisms is attributable to the specific adsorbing characteristics of the bacterial cell onto the ITO surface.

Antibacterial effects of the TiO_2 powder under light irradiation can be interpreted in terms of the photocatalytic effect of TiO_2. We reported that the antibacterial function of TiO_2 powder is originated not only in the photocatalytic effect but also in the adsorptive effect.[7] Here, in this experiment, two different types of TiO_2 powder were used for antibacterial effect investigation.

In results, four different types of behavior were observed in the antibacterial effect of TiO_2.

In the first type as observed in L. a (Fig. 2(a)) and in S. m (Fig.2(b)), both types of TiO_2 powders were effective and the viability cells by the conventional colony counting method decreased upon light irradiation.

In the second type observed in A. a (Fig. 3), the viability of bacterial cells was affected by the rutile TiO_2 (TP-3) but not by anatase (ST-41).

In the third type, shown by L. c (Fig. 48a)), S. s (Fig. 4(b)), E. c (Fig.4(c)) and S. a (Fig. 4(d)), the cells were affected by anatase type TiO_2 (ST-41).

The fourth type, shown on S. f (Fig. 5), the cells were not affected by both rutile and anatase type of TiO_2 powders.

Photocatalytic reaction studies indicated that the antibacterial effect of the TiO_2 powder was dependent upon a reduction and oxidation mechanism, namely, a photocatalytic reaction which decreased co-enzyme-A (CoA) in the microorganisms.[4] Kawai and co-workers[7,8] noticed that the antibacterial effect of TiO_2 was interpreted

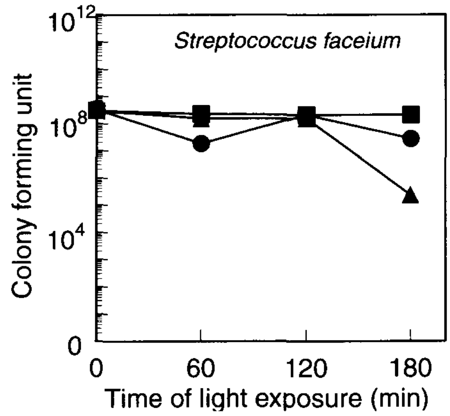

Fig.5. Changes in the viability of Streptococcus faceium with 0.1% (W/V) concentration of the powdered semiconductor TiO_2 (■; TP-3, ▲; ST-40) with light exposure time. The controls (●) were incubated without TiO_2.

図5　光照射下における酸化チタン微粒子0.1%W/V 添加電解液中での供試菌 Streptococcus faceium の活性（活動性）の経時変化　酸化チタンの種類(■; TP-3, ▲; ST-40)　酸化チタン無し(●).

by taking both the oxidation of CoA by photogenerated holes on the valence band of TiO_2 particle and the adsorptive effect into consideration. Furthermore, it was indicated that the antibacterial effect of TiO_2 powder was depended upon the pH value of the electrolyte solution. On the other hand, We reported that the antibacterial effect of TiO_2 was dependent upon variable sensitivity of bacterial cell wall to photocatalytic reaction and protection from the photocatalytic reaction depending on the ability of bacteria to adsorb to TiO_2 powder.[10-12]

In this study, it was indicated that differences in the particle size (TP-3; 1.44 μm, ST-41; 50 nm) and the kind of TiO_2 powder (rutile type and anatase type) may also influence on the mechanism of the antibacterial effect.

Therefore, it is highly possible that this cyclic voltammetry system may be used not only for the evaluation of the bacterial viability but also the classification of the bacterial strains, and further the compound of the different types of TiO_2 powder may be used for one of the materials of dental caries prevention, because of antibacterial effect.

In summary, the present electrochemical studies on cariogenic microorganisms and antibacterial effects of TiO_2 can be summarized as follows:

1) The electrochemical characteristics such as oxidation potential and oxidation current are dependent on on the kinds of microorganisms in the aqueous phosphorous buffer solution.
2) The examined microorganisms show specific adsorptive behavior on the ITO as well as TiO_2 powder.
3) The antibacterial effect of TiO_2 powder is different with the kinds of microorganisms and the type of TiO_2 powder.
4) Possibility of electrochemical classification of various cariogenic microorganisms was clarified.

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